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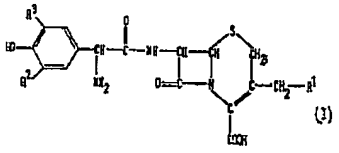
(54) ANTIBACTERIAL AGENTS AND A PROCESS FOR THE PREPARATION THEREOF

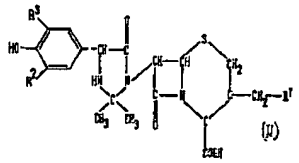
(71) We, BRISTOL-MYERS COMPANY, a corporation of the State of Delaware, United States of America, of 630 Fifth Avenue, New York, State of New York, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement: —

This invention relates to new synthetic compounds of value as antibacterial agents, as nutritional agents in animal feeds, as agents for the treatment of mastitis in cattle and as therapeutic agents in poultry and animals, including man, in the treatment of infectious diseases caused by Gram-positive and Gram-negative bacteria. According to the invention there is provided cephalosporin compounds having the formulae



(I)

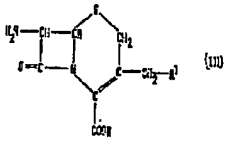
and



(II)

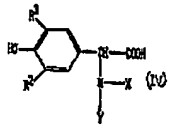
wherein R^1 is hydrogen or acetoxy and R^2 and R^3 are hydrogen or chloro, and the nontoxic pharmaceutically acceptable salts thereof.

The present invention also provides a process for the preparation of a compound of the formula I defined above, or a nontoxic pharmaceutically acceptable salt thereof; which process comprises (a) acylating a compound of the formula



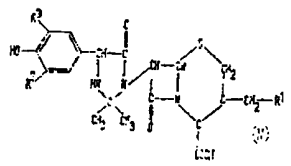
(III)

wherein R^1 is as defined above, or a carboxylic acid salt thereof (i.e., a salt formed by the reaction of a base with the carboxylic acid function, such as the potassium or triethylamine salt), with an acid of the formula



(IV)

or an acylating derivative thereof, wherein —NXY is a protected amino group in which X is hydrogen and Y is t - butoxycarbonyl, benzyloxycarbonyl or 2,2,2 - trichloroethoxycarbonyl, or a like protecting group, or X and Y when taken together represent the 2 - hydroxy-1 - naphthylmethylene group, or a like protecting group, in an inert solvent, at a temperature below 0° C., and (b) subsequently removing the protecting group to produce the desired compound, or a nontoxic, pharmaceutically acceptable salt thereof. If desired, said compound may be reacted with acetone, at a pH of from 5 to 9, at a temperature of from −20° C. to 50° C., preferably in the absence of a major amount of water, to produce a compound of the formula

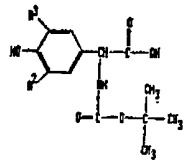


wherein R^1, R^2 and R^3 are defined above, or a nontoxic, pharmaceutically acceptable salt thereof.

For purposes of nomenclature it may be pointed out that the D-(-) isomer of the compound of formula I in which both R^2 and R^3 are chloro and R^1 is acetoxy is named 7 - [D-(−) - α - amino - α - (3,5 - dichloro - 4 - hydroxyphenyl) - acetamido]cephalosporanic acid. The D-(-) isomer of the compound of Formula I wherein R^2 and R^3 are both chloro and R^1 is hydrogen may be named either 7-[D - (-) - α - amino - α - (3,5 - dichloro - 4-hydroxyphenyl) - acetamido] - decaphalosporanic acid or 7 - [D - (-) - α - amino - α-(3,5 - dichloro - 4 - hydroxyphenyl) - acetamido] - 3 - methyl - 3 - cephem - 5 - carboxylic acid. The D-(-) isomer of the compound of Formula II in which R^2 and R^3 are chloro and R^1 is acetoxy is named 7 - [D - (-)-2,2 - dimethyl - 4 - (3,5 - dichloro - 4 - hydroxyphenyl) - 5 - oxo - 1 - imidazolidinyl] cephalosporanic acid. The D-(-) isomer of the compound of Formula II in which R^2 and R^3 are chloro and R^1 is hydrogen may be named either 7 - [D - (-) - 2,2 - dimethyl - 4 -(3,5-dichloro - 4 - hydroxyphenyl) - 5 - oxo - 1-imidazolidinyl] - decephalosporanic acid or 7-[D - (-) - 2,2 - dimethyl - 4 - (3,5 - dichloro-4 - hydroxyphenyl) - 5 - oxo - 1 - imidazolidinyl] - 3 - methyl - 3 - cephem - 5 - carboxylic acid.

The nontoxic, pharmaceutically acceptable salts referred to above include, for example, (1) non-toxic pharmaceutically acceptable salts of the acidic carboxylic acid group such as the sodium, potassium, calcium, aluminium and ammonium salts and nontoxic substituted ammonium salts with amines such as tri(lower)-alkylamines, procaine, dibenzylamine, N-benzyl - beta - phenethylamine, 1 - ephenamine, N,N′ - dibenzylethylenediamine, dehydroabietylamine, N,N′ - bisdehydroabietylethylenediamine, N - (lower)alkylpiperidines, such as N - ethylpiperidine and other amines which have been used to form salts of benzylpenicillin; and (2) nontoxic pharmaceutically acceptable acid addition salts (i.e., salts of the basic nitrogen) such as (a) the mineral acid addition salts such as hydrochloride, hydrobromide, hydroiodide, sulfate, sulfamate, sulfonate, phosphate, etc. and (b) the organic acid addition salts such as the maleate, acetate, citrate, tartrate, oxalate, succinate, benzoate, fumarate, malate, mandelate, ascorbate, β-naphthalene sulfonate and, p - toluenesulfonate. Also included are the easily hydrolyzed esters or amides of such acids which may be converted to the free acid form by chemical or enzymatic hydrolysis. As used herein the term "(lower)alkyl" is defined as including straight and branched chain saturated hydrocarbon radicals having from 1 to 10 carbons inclusive.

In the process of this invention, 7 - aminocephalosporanic acid or 7 - aminodecephalosporanic acid is acylated with an acid of the formula



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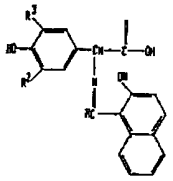

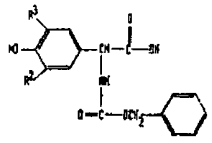

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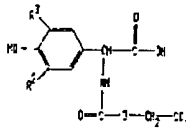


or an acylating derivative thereof. The acylating acid is preferably in the form of its mixed acid anhydride, but its functional equivalent as an acylating agent for a primary amine may also be utilized. Preferred mixed anhydrides include particularly the mixed anhydrides prepared from stronger acids such as the lower aliphatic monoesters of carbonic acid, of alkyl and aryl sulfonic acids and of more hindered acids such as diphenylacetic acid. The functional equivalents include the corresponding carboxylic chlorides, bromides and then acid anhydrides. In addition, an acid azide or an active ester or thioester (e.g., with p - nitrophenyl, 2,4 - dinitrophenal, thiophenol, thioacetic acid) may be used, or the free acid itself may be coupled with 7 - aminocephalosporanic

acid or 7 - aminodecephalosporanic acid after first reacting said free acid with N,N' - dimethylchloroformiminium chloride [cf. Great Britain 1,008,170 and Novak and Weichet, Experientia XXI/6, 360 (1965)] or by the use of enzymes or of an N,N' - carbonyldiimidazole or an N,N' - carbonylditriazole [cf. South African Patent Specification 63/2684] of a carbodiimide reagent [especially N,N'-dicyclohexylcarbodiimide, N,N' - diisopropylcarbodiimide or N - cyclohexyl - N' - (2-morpholinoethyl)carbodiimide; cf. Sheehan and Hess, J. Amer. Chem. Soc 77, 1067, (1955)], or of alkynylamine reagent [cf. R. Buijle and H. G. Viehe, Angew. Chem. International Edition 3, 582 (1964)], or of a ketenimine reagent [cf. C. L. Stevens and M. E. Monk, J. Amer. Chem. Soc. 80, 4065 (1958)] or of an isoxazolium salt reagent [cf. R. B. Woodward, R. A. Olofson and H. Mayer, J. Amer. Chem. Soc 83, 1010 (1961)]. Another equivalent of the acid chloride is a corresponding azolide, i.e. an amide of the corresponding acid whose amide nitrogen is a member of a quasiaromatic five-membered ring containing at least two nitrogen atoms, i.e. imidazole, pyrazole, the triazoles, benzimidazole, benzotriazole and their substituted derivatives. As an example of the general method for the preparation of an azolide, N,N' - carbonyl - diimidazole is reacted with a carboxylic acid in equimolar proportions at room temperature in tetrahydrofuran, chloroform, dimethylformamide or a similar inert solvent to form the carboxylic acid imidazolide in practically quantitative yield with liberation of carbon dioxide and one mole of imidazole. Dicarboxylic acids yield diimidazolides. The by-product, imidazole, precipitates and may be separated and the imidazolide isolated, but this is not essential. The methods for carrying out these reactions to produce a cephalosporin and the methods used to isolate the cephalosporin so-produced are well-known in the art (cf. U.S. Patents Nos. 3,079,314; 3,117,126 and 3,129,224 and British Patents Nos. 932,644; 957,570 and 959,054).

The blocking group is then removed to form the products of the present invention, e.g., the t - butoxycarbonyl group is removed by treatment with formic acid, the benzyloxycarbonyl group is removed by catalytic hydrogenation, the 2 - hydroxy - 1 - naphthylmethylene group is removed by acid hydrolysis and the 2,2,2-trichloroethoxycarbonyl group is removed by treatment with zinc dust in glacial acetic acid. Obviously other functionally equivalent blocking groups for an amino group can be used and such groups are considered within the scope of this invention.

The inert solvents useful in the acylation step described above are well-known to those skilled in the art, and include such solvents as tetrahydrofuran, dimethylformamide, methylene chloride, diethyl ether, acetone, chloroform, methyl isobutyl ketone, ethyl acetate and the dimethyl ethers of ethylene glycol and diethylene glycol. The acylation should be conducted at a temperature below 0° C. and is preferably conducted at or below −25° C.

The compounds of Formula II are prepared by reaction of acetone with the corresponding cephalosporin compound of Formula I. Although some reaction will occur no matter what molar proportion of reactants is used, it is preferable in order to obtain maximum yields to use a molar excess of the acetone and the latter may well be used as the reaction solvent. Water is split off during the reaction and it is thus preferable not to have a major amount of water in the reaction medium. The pH of the reaction mixture should be from 5 to 9 and preferably on the alkaline side. The pH may be adjusted to within this range, if necessary, by the addition of an alkaline material such as, for example, sodium hydroxide, sodium carbonate, potassium hydroxide, potassium carbonate, ammonium hydroxide, ammonium carbonate, or organic amines (e.g., triethylamine). The temperature during the reaction is not critical. The reaction will proceed satisfactorily at room temperature and may be hastened by heating.

The carbon bearing the free amino group in the compounds of Formula I is an asymmetric carbon atom and thus the compounds of Formula I and II can exist in two optically active forms (the D- and L- diastereoisomers), as well as in a mixture of the two optically active forms (the racemic mixture).

The compounds of the present invention are useful in the treatment of infections caused by Gram-positive bacteria, including particularly the resistant strains of bacteria and Gram-negative bacteria, e.g., penicillin-resistant strains of *Staphylococcus aureus* (*Micrococcus pyogenes* var. aureus). In addition, the compounds of the present invention are orally absorbed.

In the treatment of bacterial infections in man, the compounds of this invention are administered orally or parenterally, in accordance with conventional procedures for antibiotic administration, in an amount of from 5 to 60 mg./kg./day and preferably 20 mg./kg./day in divided dosage, e.g. three or four times a day. They are administered in dosage units containing, for example, 125 or 250 or 500 mg. of active ingredients with suitable physiologically acceptable carriers or excipients. The dosage units can be in the form of liquid preparations such as solution, dispersions or emulsions or in solid form such as tablets or capsules.

Thus the invention includes a pharmaceutical composition comprising a compound of formula I or formula II, or a salt thereof, and a carrier or diluent.

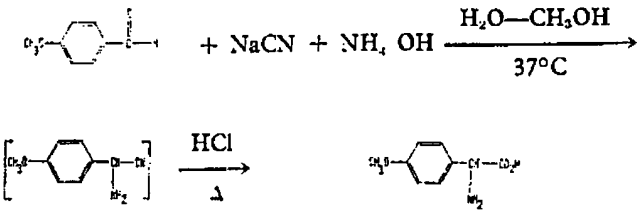
The invention further includes a method of treating animals including poultry, but ex-

cluding man, for diseases caused by Gram-positive or Gram-negative bacteria, which method comprises administering to the animal an effective dose of a compound of formula I or formula II, or a salt thereof, or a pharmaceutical composition as hereinbefore defined.

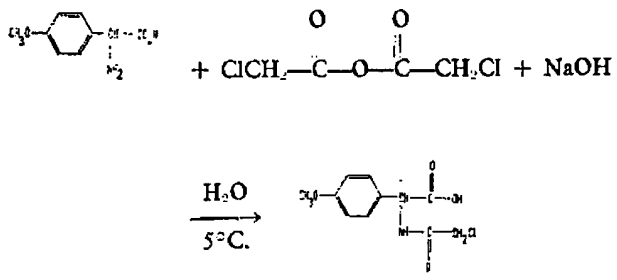
Preparation of Starting Materials

D - (−) - 2 - (p - Hydroxyphenyl)glycine used as a starting material for the preparation of the compounds of this invention is prepared according to the following reaction scheme starting with anisaldehyde.

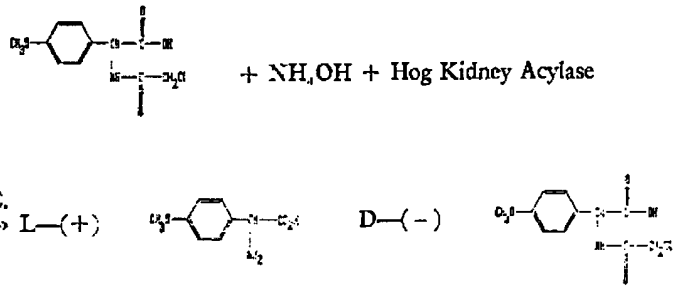
I.



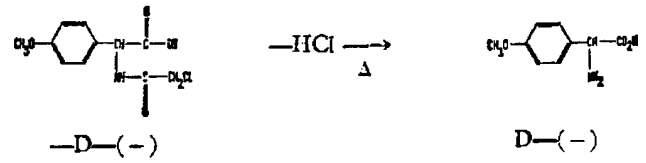
II.



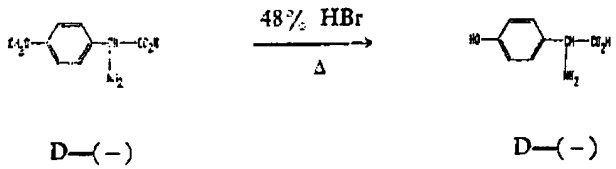
III.



IV.



V.



I. dl - 2 - (p - Methoxyphenyl) - glycine-

To a stirred solution of 19.6 g. (0.4 mole) of NaCN in 80 ml. of H_2O was added 23.6 g. (0.450 mole) of NH_4Cl and 20 ml. of conc. NH_4OH followed by 54.5 g. (0.4 mole) of anisaldehyde in 160 ml. of methanol and the temperature maintained at 37° C. for two hours. The methanol was then removed *in vacuo* and the remaining mixture extracted with two 150 ml. portions of methyl isobutyl ketone (MIBK) and combined. The combined MIBK extracts were washed once with 30 ml. of H_2O and then 240 ml. of 6NHCl added with good mixing and the MIBK was removed *in vacuo*. The resulting slurry was heated at reflux (now in solution) for two hours. One hundred ml. of H_2O was added to the hot solution and then 8 g. of decolorizing carbon added and after ten minutes at gentle reflux the carbon was filtered off and washed with 50 ml. of hot water. The combined filtrates (hot) were stirred and treated with conc. NH_4OH until pH 5—6 was obtained (pH paper). The slurry was then cooled to 5° C. and after one hour the crystals were filtered off and washed with two 100 ml. portions of water. The damp cake was then slurried in 250 ml. of water and 50% NaOH added slowly until the product dissolved. Two 300 ml. ether extracts were then taken and discarded. The pH was then adjusted to 5.5 with 6NHCl with cooling. After one hour the product was filtered off, washed with 3 × 100 ml. H_2O and air dried. Yield 40 g.; dec. 244° C. with sublimation at 230° C.

II. dl - 2 - (p - Methoxyphenyl) - N-(chloroacetyl) - glycine

To a stirred suspension of 36 g. (0.2 mole) of dl - 2 - (p - methoxyphenyl) - glycine in 500 ml. of H_2O was added 8 g. (0.2 mole) of NaOH pellets and when a clear solution was obtained the solution was cooled to 5° C. and with vigorous stirring 68.2 g. (0.4 mole) of chloroacetic anhydride (warm) was added all at once. Then a solution of 16 g. (0.4 mole) of NaOH in 100 ml. of H_2O was added over a 10 to 15 minute period. More 20% NaOH was added as needed to keep the pH at about 9 for a 1.5 hour period. Next, the pH was adjusted to 2 with 40% H_3PO_4. The product crystallized immediately and was filtered off, washed with water and recrystallized from ethanol-water to give 38 g. of product melting at 182°—183° C.

Anal. Calcd. for $C_{11}H_{12}ClNO_4$:
C, 51.21; H, 4.69;
Found: C, 51.49; H, 4.90.

III. D - (−) - 2 - (p - Methoxyphenyl) - N-(chloroacetyl)glycine and L - (+) - 2 - (p-Methoxyphenyl) - glycine.

To 800 ml. of H_2O stirred at 37° C. was added 38 g. (0.148 mole) of dl - 2 - (p-methoxyphenyl) - N - (chloroacetyl) - glycine and NH_4OH added dropwise until pH 7.8 was obtained. To the resulting solution was added 2 g. of Hog Kidney Acylase (Sigma Chemical Company) and stirring continued at 37° C. (internal) for 21 hours. The solids containing crude L - (+) - 2 - (p - methoxyphenyl) - glycine were then filtered off and washed with 2 × 100 ml. H_2O and the pH of the combined filtrates adjusted to 4—5 with glacial acetic acid. This solution was heated on the steam bath for 30 min. with 5 g. of decolorizing carbon and then filtered. The carbon cake was washed with 50 ml. of warm water and the combined filtrates cooled and acidified to pH 2 with 40% H_3PO_4. After one hour cooling at 0° C., the crystalline product was filtered off and washed with cold water (3x) and air dried. The yield was 16 g. of D - (−) - 2 - (p - methoxyphenyl) - N-(chloroacetyl) - glycine and when a second run using 5x the above amounts were used a yield of 83 g. (87% yield) was obtained, m.p. 170°—171° C.;

$[\alpha]_D^{25°\,C.}$ −193° (C=1%, ethanol)

Analysis for $C_{11}H_{12}ClNO_4$:
Calculated: C, 51.21; H, 4.69
Found: C, 51.50; H, 4.99.

When the solids containing crude L - (+)-2 - (p - methoxyphenyl)glycine are treated with hot 3 NHCl (200 ml.) and carbon followed by filtration and pH adjusment to 5.5 there is obtained 6 g. (first run) of pure L - (+) - 2-(p - methoxyphenyl)glycine.

$[\alpha]_D^{25°\,C.}$ +150.4° (C=1%, 1NHCl)

IV. D - (−) - 2 -)p - Methoxyphenyl)glycine.

The 16 g. of D - (−) - 2 - (p - methoxyphenyl) - N - (chloroacetyl)glycine was refluxed 1.5 hours in 170 ml. of 2 NHCl. The resulting clear solution was filtered and cooled at 5° C. and the pH adjusted to 5.5 with NH_4OH. The product was then filtered off after cooling 30 minutes and washed with 3 × 25 ml. of cold water. The dried material D-(−) - 2 - (p - methoxyphenyl)glycine weighed *9.5 g.* A *second* run gave *54 g.* using the 83 g. of starting material from III.

$[\alpha]_D^{25°\,C.}$ −149.9° (C=1%, 1 NHCl) (first run)

$[\alpha]_{D}^{25^\circ C.}$ -148.1° (C=1%, 1 NHCl)
(second run)
Analysis for $C_9H_{11}NO_3$:
Calculated: C, 59,67; H, 6.13; N, 7.74
Found: C, 59.38; H, 6.16; N, 8.00.

V. D - (−) - 2 - (p - Hydroxyphenyl)glycine.

A mixture of 1.81 g. (0.01 mole) of D - (−) - 2 - (p - methoxyphenyl)glycine, ($[\alpha]_{D}^{25^\circ C.}$ -149.9° (C=1%, 1NHCl) and 10 ml. of 48% HBr was heated at gentle reflux for 2 hours. The resulting solution was concentrated at reduced pressure at 30° C. to a wet solid. A minimum amount of water (20° C) was added to dissolve the HBr salt and with cooling NH_4OH was added to pH 5. The resulting thick gel which precipitated was warmed to 50° C. and when solution was nearly obtained a different crystalline form began to precipitate. Upon cooling 30 minutes at 0°—5° C. there was obtained 990 mg. of cold water washed (3 × 1 ml.) and air dried material, D - (−) - 2 - (p - hydroxyphenyl) glycine.

$[\alpha]_{D}^{25^\circ C.}$ -161.2° (C=1%, 1NHCl)
dec. pt. 223.° C.

A second run using 20x the above amounts gave 24.5 g. of material.

$[\alpha]_{D}^{25^\circ C.}$ -153° (C=1%, 1 NHCl)
Analysis for $C_9H_{11}NO_3$:
Calculated: C, 57.49; H, 5.43; N, 8.39
Found: C, 57.41; H, 5.67; N, 8.39.

VI. D - (−) - α - (p - Hydroxyphenyl) - α - (t - butoxy - carbonylamino)acetic acid.

To a stirred suspension of 8.35 g. (0.05 mole) of D - (−) - 2 - (p - hydroxyphenyl) glycine (finely ground) and 8 g. (0.2 mole) of powdered magnesium oxide in 225 ml. of 1:1 dioxane-water was added 14.3 g. (0.10 mole) of t - butoxycarbonylazide (Aldrich Chemical Company Inc.) over a 30 minute period and then stirring continued for 20 hours at 45°—50° C. The resulting turbid solution was then poured into one liter of ice water with stirring. One 600 ml. ethyl acetate extract was taken and this was washed twice with 200 ml. portions of 5% sodium bicarbonate and these aqueous solutions combined and filtered. Next, with cooling, they were acidified to pH 3 with 40% phosphoric acid under a layer of 500 ml. of ethyl acetate. This ethyl acetate extract was separated and combined with two more 100 ml. ethyl acetate extracts and dried over sodium sulfate. The ethyl acetate solution was then filtered and concentrated under reduced pressure to an oil and 100 ml. of warm benzene added. The resulting solution was filtered and scratched. There was obtained 10.8 g. of crystalline material, D-(−) - α - (p - hydroxyphenyl) - α - (t - butoxycarbonylamino)acetic acid. Infrared and NMR analysis revealed only the NH_2 group had reacted with the azide.

Anal. Calc'd for $C_{13}H_{17}NO_5$:
C, 58.43; H, 6.48; N, 5.25
Found: C, 62.46; H, 6.55; N, 4.56.

VII. D - (−) - 2 - (3 - chloro - 4 - hydroxyphenyl)glycine

To a stirred suspension of 5.01 g. (0.03 mole) of D - (−) - 2 - (p - hydroxyphenyl) glycine in 100 ml. of glacial acetic acid was bubbled in HCl gas at a vigorous rate for about 5 minutes. At first a clear solution resulted and then the hydrochloride salt crystallized out. Next, 4.45 g. (0.033 mole) of sulfuryl chloride (freshly distilled) in 25 ml. of glacial acetic acid was added, with stirring, over a 30 minute period, dropwise. The temperature was 26°—27° C. throughout the addition. After one hour stirring, 250 ml. of dry ether was added slowly and crystallization began. After 15 min. the product was filtered off, washed with dry ether and air dried. The 7 g. obtained was dissolved in 50 ml. of 1NHCl, filtered, and the pH adjusted, with cooling to 5 with conc. NH_4OH. The resulting crystalline product was filtered off after 5 min. standing, washed with two 20 ml. portions of water and 5x with acetone. The vacuum dried material weighed 4.6 g.; dec. pt. 217° C. (sharp). The NMR and IR spectra were consistent with the desired structure.

$[\alpha]_{D}^{22^\circ C.}$ -137.1° (C=1%, 1NHCl)
Anal. Calcd. for $C_9H_8ClNO_3$:
C, 47.76; H, 4.01; Cl, 17.66
Found: C, 47.16; H, 3.92; Cl, 17.96.

VIII. D - (−) - α - (3 - chloro - 4 - hydroxyphenyl) - α - (t - butoxycarbonylamino) acetic acid.

To a stirred suspension of 4.0 g. (0.02 mole) of D - (−) - 2 - (3 - chloro - 4 - hydroxyphenyl)glycine (finely ground) and 1.6 g. (0.04 mole) of powdered magnesium oxide in 50 ml. of 1:1 dioxane-water was added 5.8 g. (0.04 mole) of t - butoxy - carbonylazide (Aldrich Chemical Company Inc.) over a 30 minute period and then stirring continued for 20 hours at 45°—50° C. The resulting turbid solution was then poured into one liter of ice water with stirring. One 600 ml. ethyl acetate extract was taken and this was washed twice with 200 ml. portions of 5% sodium bicarbonate and these aqueous solutions combined and filtered. Next, with cooling, they were acidified to pH 3 with 40% phosphoric acid under a layer of 500 ml. of ethyl acetate. This ethyl acetate extract was separated and combined with two more 100 ml.

ethyl acetate extracts and dried over sodium sulfate. The ethyl acetate solution was then filtered and concentrated under reduced pressure to an oil and 100 ml. of warm benzene added. The resulting solution was filtered. After removing the solvent *in vacuo* there was obtained 6 g. of an amorphous froth D - (−)- α - (3 - chloro - 4 - hydroxyphenyl) - α - (t-butoxycarbonylamino) acetic acid. Infrared and NMR analysis revealed only the NH_2 group had reacted with the azide.

IX. D - (−) - 2 - (3,5 - dichloro - 4 - hydroxyphenyl) - glycine.

To a stirred suspension of 5.01 g. (0.03 mole) of D - (−) - 2 - (4 - hydroxyphenyl)-glycine in 100 ml. of glacial acetic acid was bubbled in HCl gas at a vigorous rate for about 5 minutes. At first a clear solution resulted and then the hydrochloride salt crystallized out. Next 9.0 g. (0.067 mole) of sulfuryl chloride (freshly distilled) in 25 ml. of glacial acetic acid was added, with stirring, over a 30 minute period, dropwise. The temperature was 26°—27° C. throughout the addition. After the sulfuryl chloride addition, the slurry was heated to 70° C. for 30 minutes and then stirred at ambient temperature for two hours. Then 250 ml. of dry ether was added slowly and crystallization began. After 15 min. the product was filtered off, washed with dry ether and air dried. The 7 g. obtained was dissolved in 100 ml. of 1NHCl, filtered, and the pH adjusted, with cooling to 5 with conc. NH_4OH. The resulting crystalline product was filtered off after 5 min. standing, washed with two 20 ml. portions of water and 5x with acetone. The vacuum dried material weighed 4.5 g.; dec. pt. 210° C. (sharp). The NMR and IR spectra were consistant with the desired structure.

$[\alpha]_D^{22°\ C.}$ −126.3° (C=1%, 1NHCl)

Anal. Calcd. for $C_8H_7Cl_2NO_3$:
C, 40.68; H, 2.99; Cl, 30.04
Found: C, 41.85; H, 3.22; Cl, 27.90.

X. D - (−) - α - (3,5 - dichloro - 4 - Hydroxyphenyl) - α - (t - butoxycarbonylamino)acetic acid.

To a stirred suspension of 4.2 g. (0.0178 mole) of D - (−) - 2 - (3,5 - dichloro - 4-hydroxyphenyl)glycine (finely ground) and 1.6 g. (0.04 mole) of powdered magnesium oxide in 50 ml. of 1:1 dioxane - water was added 5.8 g. (0.04 mole) of t - butoxy - carbonyl-azide (Aldrich Chemical Company Inc.) over a 30 minute period and then stirring continued for 20 hours at 45° —50° C. The resulting turbid solution was then poured into one liter of ice water with stirring. One 600 ml. ethyl acetate extract was taken and this was washed twice with 200 ml. portions of 5% sodium bicarbonate and these aqueous solutions combined and filtered. Next, with cooling, they were acidified to pH 3 with 40% phosphoric acid under a layer of 500 ml. of ethyl acetate. This ethyl acetate extract was separated and combined with two more 100 ml. ethyl acetate extracts and dried over sodium sulfate. The ethyl acetate solution was then filtered and concentrated under reduced pressure to an oil and 100 ml. of warm benzene added. The resulting solution was filtered. After stripping the solvent there was obtained 5 g. of amorphous material, D - (−) - α - (3,5-dichloro - 4 - hydroxyphenyl) - α - (t - butoxycarbonylamino) acetic acid. Infrared and NMR analysis revealed only the NH_2 group had reacted with the azide.

Following is a description by way of example to further illustrate starting materials, intermediates, compounds and process in accordance with the invention.

Examples I, IV, VIII and XIII are illustrative of the starting materials and intermediates employed in the processes of the present invention.

EXAMPLE 1

7 - [D - (−) - α - (t - butoxycarbonylamino)- α - (p - hydroxyphenyl) - acetamido]cephalosporanic acid

a) To a stirred solution of 5.35 g. (0.02 mole) of D - (−) - α - (p - hydroxyphenyl)- α - (t - butoxycarbonylamino)acetic acid, 2.02 g. (0.02 mole) of 2,6 - lutidine and 50 ml. of tetrahydrofuran at −10° C. was added all at once 2.16 g. of (0.02 mole) ethyl chloroformate. After 20 minutes an ice cold solution of 5.44 g. (0.02 mole) of 7 - amino - cephalosporanic acid, 5 g. of sodium bicarbonate in 50 ml. of water was added, all at once with vigorous stirring. The temperature was kept at or below 0° C. for 10 minutes and between 0° C. and +10° C. for 90 minutes. Next, 100 ml. of water was added and the tetrahydrofuran removed *in vacuo* at 20° C. One 200 ml. ether extract was taken and discarded. The aqueous phase was layered with 200 ml. of methyl isobutyl ketone and cooled and stirred while being acidified to pH 2. The methyl isobutyl ketone layer was washed with two 100 ml. portions of water, dried briefly over sodium sulfate filtered and treated with 7 ml. (0.02 mole) of 50% NaEH (Sodium 2 - ethylhexanoate in n-butanol). An oily precipitate separated and slowly crystallized. After one hour, they were filtered off, washed with methyl isobutyl ketone and air dried. After further drying over phosphorus pentoxide (vacuum) there was obtained 5.24 g. dec. slowly above 100° C. The infrared and NMR spectra were consistent with the structure of sodium 7 - [D - (−) - α - (t - butoxycarbonylamino) α - (p - hydroxyphenyl) - acetamido]cephalosporanate.

The free acid 7 - [D - (—) - α - (t - butoxycarbonylamino) - α - (p - hydroxyphenyl)-acetamido]cephalosporanic acid was obtained as an amorphous gum by extracting an acidic aqueous solution with ethyl acetate and concentrating under reduced pressure.

b) (Alternate Procedure)

To a stirred solution of 5.35 g. (0.02 mole) of D - (—) - α - p - hydroxyphenyl) - α-(t - buoxycarbonylamino)acetic acid, 100 ml. of tetrahydrofuran, and 2.8 ml. (0.02 mole) of triethylamine at —40° C. was added dropwise 3.64 g. (0.02 mole) of trichloroacetic acid in 25 ml. of tetrahydrofuran over a 20 minute period. Next, after an additional 15 minutes; a solution of 5.44 g. (0.02 mole) of 7 - aminocephalosporanic acid 5.6 ml. (0.04 mole) of triethylamine in 300 ml. of methylene chloride precooled to —40° C. was added all at once and the temperature maintained at —40° C. to —30° C. for 45 minutes. The mixture was then concentrated under reduced pressure at 20° C. to an oil. This was taken up in 200 ml. of 2% aqueous sodium bicarbonate and 200 ml. of ether. The ether layer was discarde dand the aqueous phase layered with 200 ml. of ethyl acetate and with cooling and stirring, the mixture acidified to pH 3. The ethyl acetate layer was then separated and washed twice with water, dried briefly over sodium sulfate, filtered and evaporated to an oil under reduced pressure at 20° C. Five hundred ml. of ether was then added and a small amount of insoluble material filtered off. The ether solution was then concentrated to about 200 ml. and then 200 ml. of Skellysolve B (petroleum ether) was added. The precipitate which formed was separated by filtration and consisted of the desired product, 7 - [D - (—) - α - (t - butoxycarbonylamino)-α - (p - hydroxyphenyl) - acetamido]cephalosporanic acid.

Yield = 6 g.

Example 2

7 - [D - (—) - α - amino - α - (p - hydroxyphenyl) - acetamido]cephalosporanic acid

7 - [D - (—) - α - (t - butoxycarbonylamino) - α - (p - hydroxyphenyl) - acetamido] cephalosporanic acid (6 g.) was dissolved in 100 ml. of 50% aqueous formic acid and stirred at 40° C. for 3 hours. The solution was then treated with 1 g. of decolorizing carbon and filtered. The filtrate was concentrated to a viscous oil at 20° C. under reduced pressure. The last traces of formic acid were removed by adding 300 ml. of toluene and removing same under reduced pressure at 20° C. The resulting glass was triturated with 400 ml. of ethyl acetate to which 5 ml. of water had been added. A semi-crystalline solid formed which was filtered off and vacuum dried over phosphorus pentoxide. The product, 7 - [D - (—)-α - amino - α - (p - hydroxyphenyl) - acetamido] - cephalosporanic acid, weighed 1.8 g. and had a decomposition point of 260° C. with darkening above 150° C. Infrared and NMR spectra were consistent with the structure.

Anal. Calc'd. for $C_{18}H_{19}N_3O_7S$:
C, 51.07; H, 4.55
Found: C, 51.58; H, 5.12.

This product is found to inhibit *Staphylococcus aureus* Smith at concentration of 5.0 mcg./ml., *Streptococcus pyrogenes* at a concentration of 0.08 mcg./ml. *Staphylococcus aureus* BX—1633—2 (a strain resistant to benzylpencillin) at a concentration of 6.2 mcg./ml., *Escherichia coli* Juhl at a concentration of 6.2 mcg./ml., *Salmonella enteritidia* at a concentration of 6.2 mcg./ml., and *Diplococcus pneumoniae* at a concentration of 2.5 mcg./ml., to exhibit upon intramuscular injection in mice a CD_{50} against *Staph. aureus* Smith of 0.2 mg./kg. against *Staph aureus* BX—1633—2 of 25—36 mg./kg., against *Salmonella enteritidia* of 4 mg./kg., against *E. coli* of 10 mg./kg., and against *Diplococcus pneumoniae* of 4.5 mg./kg. and to exhibit upon oral administration in mice a CD_{50} against *Diplococcus pneumoniae* of 7.0 mg./kg., against *Staph. aureus* Smith of 3.0 mg./kg., against *Staph. aureus* BX—1633—2 of 45—62 mg./kg., against *Salmonella enteritidia* of 76 mg./kg., against *Klebsiella pneumoniae* of 40 mg/kg. and against *E. coli* of 40 mg./kg.

A comparison was made of the blood levels obtained in mice upon oral administration of 7 - [D - (—) - α - amino - α - (p - hydroxyphenyl) - acetamido]cephalosporanic acid with the blood levels obtained with cephaloglycine (7 - [D - (—) - α - aminophenyl - acetamido] cephalosporanic acid. In the test twelve mice were dosed orally with 0.24 m. moles/kg. of each compound. The following are the average blood levels obtained:

Time (hours)	Blood Levels (mg./ml.) 7-[D-(-)-α-amino-α-(p-hydroxyphenyl)-acetamido] cephalosporanic acid	cephaloglycine
0.5	8.7	2.7
1.0	5.8	2.0
2.0	3.6	2.1
3.5	1.8	1.6

At each time the blood level obtained with 7-[D - (−) - α - amino - α - (p - hydroxyphenyl) - acetamido]cephalosporanic acid is higher than that obtained with cephaloglycine.

EXAMPLE 3

7 - [D - (−) - 2,2 - Dimethyl - 4 - (p - hydroxyphenyl) - 5 - oxo - 1 - imidazolidinyl] cephalosporanic acid

A solution of 2.1 g. (0.005 mole) of 7 - D-(−) - α - amino[α - (p - hydroxyphenyl)-acetamido]caphalosporanic acid, 0.7 ml. (0.005 mole) of triethylamine in 50 ml. of methanol was obtained by stirring for 15 minutes at room temperature (22° C.). To this was added 50 ml. of acetone and stirring continued for 5 hours. The solution was then concentrated to an oil at 20° C. under reduced pressure. Twenty-five ml. of water and 50 ml. of ethyl acetate was added and the pH adjusted to 3 with 40% H_3PO_4. The aqueous layer was saturated with NaCl and the mixture shaken. The ethyl acetate layer was separated and dried briefly over Na_2SO_4, filtered and concentrated to dryness at 20° C. under reduced pressure. The resulting solid precipitate was removed by ether trituration and filtration. After drying over P_2O_5 under vacuum there was obtained 310 mg. with a decomposition point of 150°—250° C. (slowly). Infrared and NMR spectra were consistent with the desired structure.

Anal. Calcd for $C_{21}H_{23}N_3O_7S.H_2O$:
C, 52.61; H, 5.26
Found: C, 52.29; H, 5.48.

This product is found to inhibit *Staphylococcus aureus* Smith at a concentration of 2.5 mcg./ml., *streptococcus pyogenes* at a concentration of 0.08 mcg./ml., *Staphylococcus aureus* BX—1633—2 (a strain resistant to benzylpenicillin) at a concentration of 6.2 mcg./ml., *Escherichia coli Juhl* at a concentration of 6.2 mcg./ml., and *Diplococcus pneumoniae* at a concentration of 1.2 mcg./ml., and to exhibit upon oral administration in mice a CD_{50} against *Staph. aureus* Smith of 3.0 mg./kg. and against *Staph. aureus* BX—1633—2 of 70 mg./kg.

A comparison was made of the blood levels obtained in mice upon oral administration of 7 - [D - (−) - 2,2 - dimethyl - 4 - (p - hydroxyphenyl) - 5 - oxo - 1 - imidazolidinyl] cephalosporanic acid with the blood levels obtained with cephaloglycine. In this test twelve mice were dosed orally with 0.24 m. moles/kg. of each compound. The following are the average blood levels obtained:

Time (hours)	Blood Levels (mg./ml.) 7-[D-(-)-2,2-dimethyl-4(p-hydroxyphenyl)-5-oxo-1-imidazolidinyl] cephalosporanic acid	cephaloglycine
0.5	3.4	2.7
1.0	3.5	2.0
2.0	2.6	2.1
3.5	1.4	1.6

At each time except 3.5 hours the blood level obtained with D - (−) - 7 - [2,2 - dimethyl - 4(p - hydroxyphenyl - 5 - oxo - 1-imidazolidinyl]cephalosporanic acid is higher than that obtained with cephaloglycine.

EXAMPLE 4

D - (−) - α - carbobenzoxyamino - α - (4-carbobenzoxyoxyphenyl)acetic acid

To a stirred suspension of 5.01 g. (0.03 mole) of D - (−) - 2 - (p - hydroxyphenyl) glycine in 100 ml. of water at 22° C. (room temperature) was added 1.2 g. (0.03 mole) of sodium hydroxide pellets. A clear solution resulted. The stirred solution was cooled to 0° C. and 2.4 g. (0.06 mole) of NaOH pellets were added. When they had dissolved 13.6 g. (0.08 mole) of carbobenzoxy chloride was added all at once with vigorous stirring. After 30 min. at 0° C. to 5° C. the pH was 7 and a few drops of 50% NaOH—H_2O was added to keep the pH at 8—9 during another 30 min. Three hundred ml. of H_2O was then added and the resulting slurry was transferred to a separatory funnel and 500 ml. of ether added. After shaking, the ether layer was discarded and the aqueous layer and solids combined with 300 ml. of ethyl acetate and the mixture acidified with shaking to pH 2 with 6NHCl. The ethyl acetate phase was combined with two more ethyl acetate extracts and washed with two 100 ml. portions of water, two 300 ml. portions of saturated Na_2SO_4 solution and filtered. Upon concentrating under reduced pressure to an oil the product crystallized. The material was recrystallized from benzene-Skellysolve "B" (pet. ether) to give 8.9 g. of material with a melting point of 101°—102° C. The infrared and NMR spectra were consistent with the desired structure.

Anal. Calcd. for $C_{24}H_{21}NO_7$:
C, 66.21; H, 4.88; N, 3.22
Found: C, 67.93; H, 5.24; N, 3.07.

EXAMPLE 5

7 - [D - (−) - α - amino - α - (p - hydroxyphenyl) - acetamido]cephalosporanic acid

D - (−) - α - carbobenzoxyamino - α - (4-carbobenzoxyoxyphenyl) - acetic acid (0.344 mole), is dissolved in 100 mls. of dimethylformamide. There is then added 2,6 - lutidine (3.7 gms.; 0.0244 mole) and the solution is cooled to 5° C. in an ice bath. Ethyl chloro formate (3.72 gms.; 0.0344 mole) is added to the cool solution over a period of five minutes. The mixture is stirred for 15 minutes and a solution of 7 - aminocephalosporanic acid (0.395 mole) in 70 mls. of water and 20 mls. of 2, 6- lutidine is added. The solution is stirred in the ice bath for 15 minutes, diluted with 500 mls. of water and extracted twice with ether. The ether extract is discarded. The pH of the solution is lowered to 2 by the addition of dilute H_2SO_4 and the product is extracted into ether. The ether extract is washed with water and the product is extracted into dilute Na_2CO_3. This extract has a pH of 7.5 and a volume of about 300 mls. It is then shaken with 7 gms. of 30% palladium on celite for 20 minutes under an atmosphere of hydrogen at a pressure of 50 p.s.i. The volume of the solution is doubled by the addition of water and the pH is lowered by 2 by the addition of dilute H_2SO_4. The catalyst is then removed by filtration and the filtrate is extracted with a mixture of 150 mls. of methyl isobutyl ketone and 8 gms. of aerosol O.T. The extract is dried over anhydrous Na_2SO_4 and neutralized to pH 4.5 by the addition of triethylamine and an amorphous solid is collected by filtration and slurried with 20 mls. of water. A crystalline solid is formed which is collected and dried *in vacuo* over P_2O_5. The product, 7 - [D - (−) - α - amino - α - (p-hydroxyphenyl) - acetamido]cephalosporanic acid, is found to contain the β - lactam structure as shown by infrared analysis.

EXAMPLE 6

7 - [D - (−) - α - amino - α - (p - hydroxyphenyl) - acetamido] - decephalosporanic acid.

When in Example 1, 7 - aminocephalosporanic acid is replaced by an equimolar amount of 7 - aminodecephalosporanic acid there is obtained 7 - [D - (−) - α - (t - butoxycarbonylamino) - α - (p - hydroxyphenyl) - acetamido] - decephalosporanic acid. Substitution in Example 2 of an equimolar amount of this compound for 7 - [D- (−) - α - (t - butoxycarbonylamino) - α - (p - hydroxyphenyl)-acetamido] - cephalosporanic acid produces the product 7 - [D - (−) - α - amino - α-(p - hydroxyphenyl) - acetamido] - decephalosporanic acid.

This product is found to inhibit *Staphylococcus aureus* Smith at a concentration of 0.001 per cent by weight.

EXAMPLE 7

7 - [D - (−) - 2,2 - dimethyl - 4 - (p - hydroxyphenyl) - 5 - oxo - 1 - imidazolidinyl] - decephalosporanic acid.

When in Example 3, 7 - [D - (−) - α-amino - α - (p - hydroxyphenyl) - acetamido]-cephalosporanic acid is replaced by an equimolar amount of 7 - [D - (−) - α - amino-α - (p - hydroxyphenyl) - acetamido] - decephalosporanic acid there is obtained the product 7 - [D - (−) - 2,2 - dimethyl - 4 - (p - hydroxyphenyl) - 5 - oxo - 1 - imidazolidinyl]-decephalosporanic acid.

This product is found to inhibit *Staphylococcus aureus* Smith at a concentration of 0.001 per cent by weight.

EXAMPLE 8

7 - [D - (−) - α - (t - Butoxycarbonylamino) - α - (3 - chloro - 4 - hydroxyphenyl) - acetamido] - cephalosporanic acid

To a stirred solution of 4.03 g. (0.02 mole) of D - (−) - α - (2 - chloro - 4 - hydroxyphenyl) - α - (t - butoxycarbonylamino) acetic acid, 2.8 ml. of triethylamine (0.02 mole) in 100 ml. of tetrahydrofuran, was added 3.64 g. (0.02 mole) of trichloroacetyl chloride in 25 ml. of tetrahydrafuran over a 10 min. period at −40° C. (internal). After an additional 10 min. at −40° C., a pre-cooled solution at −50° C. of 5.44 g. (0.02 mole) of 7—ACA, 5.6 ml. (0.04 mole) of triethylamine in 300 ml. of CH_2Cl_2 was added all at once. The temperature was maintained at −40° to −30° C. for 30 min. and then the cooling bath was removed and after another 30 min. (T max 0° C.) the solvent was removed *in vacuo* at 20° C. The residue was taken up in 150 ml. of H_2O and 150 ml. of ether. The ether layer was discarded and the aqueous layered with 150 ml. of ethyl acetate and cooled and stirred while being acidified to pH 2.5. The ethyl acetate extract was washed with water, dried 10 min. over Na_2SO_4, filtered and conc. to an oil at 20° C. under reduced pressure. The oil was triturated until a solid ppt. with two 200 ml. portion of 1:1 by volume dry ether—Skellysolve "B" (pet. ether). The solids were filtered off and vacuum dried over P_2O_5. The yield was 7.4 g. dec. pt. 100° C., slowly. The IR and NMR were consistent with the desired structure.

Anal. Calcd. for $C_{23}H_{26}ClN_3O_9S$:
C, 49.64; H, 4.71;
Found: C, 49.50; H, 5.48.

EXAMPLE 9

7 - [D - (−) - α - Amino - α - (3 - Chloro-4 - hydroxyphenyl) - acetamido] - cephalosporanic acid

A solution of 7 g. of D - (−) - 7 - [α - (t-butoxycarbonylamino) - α - (3 - chloro - 4-hydroxyphenyl) - acetamido] - cephalosporanic acid in 200 ml. of 50% aqueous formic acid was stirred and heated at 40° C. for 3 hours and then the solvents removed *in vacuo* at 20° C. to leave a glass-like residue. This was further dried by adding 200 ml. of toluene and removing same under reduced pressure at 20° C. The residue was stirred with moist ethyl acetate until solid and the solids filtered off. Next, the solids were stirred with 95% ethanol (200 ml.) for one hour and filtered. There was obtained 2.5 g. of vacuum dried material. This was further purified and crystallized by stirring for 2 hours in a mixture of 12 ml. H_2O and 12 ml. of amberlite (Registered Trade Mark) LA—1 resin (acetate form) 25% in methyl isobutyl ketone (MIBK). The product was filtered off, washed with a little MIBK—H_2O (1:1) and finally washed with acetone. The final yield was 450 mg. dec. 100° C. slowly. The IR and NMR were consistent with the desired structure.

Anal Calcd. for $C_{15}H_{15}ClN_3O_7S$:
C, 47.37; H, 3.94;
Found: C, 47.33; H, 4.98.

This product is found to inhibit *Staphylococcus aureus* Smith at a concentration of 2.5 mcg./ml., *Streptococcus pyogenes* at a concentration of 0.08 mcg./ml., *Staphylococcus aureus* BX—1633—2 (a strain resistant to benzylpenicillin) at a concentration of 3.1 mcg./ml., *Escherichia coli* Juhl at a concentration of 6.2 mcg./ml., *Salmonella enteritidis* at a concentration of 3.1 mcg./ml., and *Diplococcus pneumoniae* at a concentration of 1.2 mcg./ml., to exhibit upon intramuscular injection in mice a CD_{50} against *Staph. aureus* Smith of 0.1 mg./kg., against *Staph. aureus* BX—1633—2 of 4—6 mg./kg., against *Salmonella enteritidis* of 2.5 mg./kg., against *E. coli* of 14 mg./kg., against *Diplococcus pneumoniae* of 2.0 mg./kg. and to exhibit upon oral administration in mice a CD_{50} against *Diplococcus pneumoniae* of 15.0 mg./kg., against *Staph. aureus* Smith of 0.6 mg./kg., *Staph. aureus* BX—1633—2 of 17—19 mg./kg., against *Klebsiella pneumoniae* of 40 mg./kg. and against *E. coli* of 37 mg./kg.

A comparison was made of the blood levels obtained in mice upon oral administration of 7 - [D - (−) - α - amino - α - (3 - chloro-4 - hydroxyphenyl) - acetamido)cephalosporanic acid with the blood levels obtained with cephaloglycine 7 - [D - (−) - α - aminophenylacetamido]cephalosporanic acid). In the test eight mice were dosed orally with 0.24 moles/kg. of each compound. The following are the average blood levels obtained:

Time (hours)	Blood Levels (mg./ml.)	
	7-[D-(-)-α-amino-α-(3-chloro-4-hydroxyphenyl)-acetamido]cephalosporanic acid	cephaloglycine
0.5	3.85	2.4
1.0	2.70	1.4
2.0	1.85	0.95
3.5	0.8	0.95

At each time except 3.5 hours the blood level obtained with 7 - [D - (—) - α - amino-α - (3 - chloro - 4 - hydroxyphenyl) - acetamido]cephalosporanic acid is higher than that obtained with cephaloglycine.

EXAMPLE 10

7 - [D - (—) - 2,2 - Dimethyl - 4 - (3-chloro - 4 - hydroxyphenyl) - 5 - oxo - 1-imidazolidinyl]cephalosporanic acid

A solution of 0.005 mole of 7 - [D - (—)-α - amino - α - (3 - chloro - 4 - hydroxyphenyl) - acetamido]cephalosporanic acid and .005 mole of triethylamine in 50 ml of acetone is stirred continuously for 5 hours. The solution is then concentrated to an oil at 20° C. under reduced pressure. Twenty-five ml. of water and 50 ml. of ethyl acetate is added and the pH adjusted to 3 with 40% phosphoric acid. The aqueous layer is saturated with NaCl and the mixture shaken. The ethyl acetate layer is separated and dried briefly over sodium sulfate, filtered and concentrated to dryness at 20° C. under reduced pressure. The resulting solid precipitate is removed by ether trituration and filtration. After drying over phosphorus pentoxide under vacuum there is the product 7 - [D - (—) - 2,2 - dimethyl-4 - (3 - chloro - 4 - hydroxyphenyl) - 5 - oxo-1 - imidazolidinyl] - cephalosporanic acid. Infrared and NMR spectra are consistent with the structure.

This product is found to inhibit *Staphylococcus aureus* Smith at a concentration of 2.5 mcg./ml., *Streptococcus pyogenes* at a concentration of 0.08 mcg./ml. *Staphylococcus aureus* BX—1633—2 (a strain resistant to benzylpencillin) at a concentration of 3.1 mcg./ml., *Escherichia coli* Juhl at a concentration of 6.2 mcg./ml., *Salmonella enteritidis* at a concentration of 3.1 mcg./ml., and *Diplococcus pneumoniae* at a concentration of 1.2 mcg./ml., to exhibit upon intra-muscular injection in mice a CD_{50} against *Staph. aureus* Smith of 0.1 mg./kg., against *Staph. aureus* BX—1633—2 of 4—6 mg./kg., *Salmonella enteritidis* of 2.5 mg./kg., against *E. coli* of 14 mg./kg. and against *Diplococcus pneumoniae* of 2.0 mg./kg. and to exhibit upon oral administration in mice a CD_{50} against *Diplococcus pneumoniae* of 15.0 mg./kg., against *Staph. aureus* Smith of 0.6 mg./kg., *Staph. aureus* BX—1633—2 of 17—19 mg./kg. and against *Klebsiella pneumoniae* of 40 mg./kg. and against *E. coli* of 37 mg./kg.

EXAMPLE 11

7 - [D - (—) - α - amino - α - (3 - chloro-4 - hydroxyphenyl) - acetamido] - decephalosporanic acid

When in Example 8, 7 - aminocephalosporanic acid is replaced by an equimolar amount of 7 - aminodecephalosporanic acid there is obtained 7 - [D - (—) - α - (t - butoxycarbonylamino) - α - (3 - chloro - 4 - hydroxyphenyl) - acetamido] - decephalosporanic acid. Substitution in Example 9 of an equimolar amount of this compound for 7 - [D - (—)-α - (t - butoxycarbonylamino) - α - (3 - chloro-4 - hydroxyphenyl) - acetamido] - cephalosporanic acid produces the product 7 - [D-(—) - α - amino - α - (3 - chloro - 4 - hydroxyphenyl) - acetamido] - decephalosporanic acid.

This product is found to inhibit *Staphylococcus aureus* Smith at a concentration of 0.001 per cent by weight.

EXAMPLE 12

7 - [D - (—) - 2,2 - dimethyl - 4 - (3-chloro - 4 - hydroxyphenyl) - 5 - oxo - 1-imidazolidinyl] - decephalosporanic acid

When in Example 10, 7 - [D - (—) - α-amino - α - (3 - chloro - 4 - hydroxyphenyl)-acetamido] - cephalophoranic acid is replaced by an equimolar amount of 7 - [D - (—) - α-amino - α - (3 - chloro - 4 - hydroxyphenyl)-acetamido]decephalosporanic acid there is obtained the product 7 - [D - (—) - 2,2-

dimethyl - 2 - (3 - chloro - 4 - hydroxyphenyl) - 5 - oxo - 1 - imidazodinyl] decephalosporanic acid.

This product is found to inhibit *Staphylococcus aureus* Smith at a concentration of 0.001 per cent by weight.

EXAMPLE 13

7 - [D - (—) - α - (t - Butoxycarbonylamino) - α - (3,5 - dichloro - 4 - hydroxyphenyl) - acetamido] - cephalosporanic acid

To a stirred solution of 4.54 g. (0.015 mole) of D - (—) - α - (3,5 - dichloro - 4 - hydroxyphenyl) - α - (t - butoxycarbonylamino)acetic acid 50 ml. of tetrahydrofuran (THF), 2.1 ml. (0.015 mole) of triethylamine (TEA) at —40° C. was added, dropwise 2.73 g. (0.015 mole) of trichloroacetylchloride in 20 ml. of THF over a 10 minute period. After another 10 minutes, a solution of 4.08 g. (0.015 mole) of 7—ACA, 4.2 ml. (0.03 mole) of TEA in 150 ml. of methylene chloride, pre-cooled to —50° C. was added all at once. The temperature was maintained at —40° C. for 30 min. and then allowed to slowly come to room temperature over a one hour period. Next, the solvents were removed, *in vacuo* at 20° C., and the residue dissolved in a mixture of 300 ml. of ether and 100 ml. of water. The aqueous phase was separated and layered with 100 ml. of ethyl acetate and stirred and cooled while being acidified to pH 2.5 with 40% H_3PO_4. The ethyl acetate extract was washed once with water, dried 10 min. over Na_2SO_4, filtered and concentrated to an oil under reduced pressure at 20° C. The oil was triturated until solid with 300 ml. of 1:1 by volume or dry ether and Skellysolve B (pet. ether). The pulverized solids were filtered off, dried *in vacuo* over P_2O_5 and weighed 3 g. dec. 110° C. slowly. The IR and NMR spectra were consistent with the desired structure.

EXAMPLE 14

7 - [D - (—) - α - Amino - α - (3,5 - dichloro - 4 - hydroxyphenyl) - acetamido] - cephalosporanic acid

A total of 2.8 g. of D - (—) - 7 - [α - (t-butoxycarbonylamino) - α - (3,5 - dichloro - 4-hydroxyphenyl) - acetamido] - cephalosporanic acid above was stirred and heated at 40° C. in 100 ml. of 50% formic acid for three hours. The solution was concentrated to a glass at reduced pressure at 20°—25° C. The product was further dried by adding 100 ml. of toluene and removing same under reduced pressure at 20° C. The final viscous glass was triturated with 150 ml. of moist ethyl acetate until a powdered solid. The material was then filtered off and vacuum dried over P_2O_5. The yield was 1.9 g. dec. 150°—230° C. slowly. The IR and NMR were consistent with the desired structure.

Anal. Calcd. for $C_{18}H_{17}Cl_2N_3O_7S$:
C, 44.08; H, 3.49;
Found: C, 43.98; H, 4.46.

This product is found to inhibit *Staphylococcus aureus* Smith at a concentration of 2.5 mcg./ml., *Streptococcus pyogenes* at a concentration of 0.08 mcg./ml., *Staphylococcus aureus* BX—1633—2 (a strain resistant to benzylpenicillin) at a concentration of 6.2 mcg./ml., *Escherichia coli* Juhl at a concentration of 12.5 mcg./ml., *Salmonella enteritidis* at a concentration of 1.6 mcg./ml., and Diplococcus pneumoniae at a concentration of 1.2 mcg./ml.

The blood levels were determined in mice upon oral administration of 7 - [D - (—) - α-amino - α - (3,5 - dichloro - 4 - hydroxyphenyl) - acetamido] cephalosporanic acid. In the test eight mice were dosed orally with 0.24 m. moles/kg. and 0.1 m. moles/kg. of the compound. The following are the average blood levels obtained:

Time (hours)	Blood Levels (mg./ml.) 7-[D-(-)-α-amino-α-(3,5-dichloro-4-hydroxyphenyl)-acetamido]-cephalosporanic acid	
	0.24 m. mols/kg.	0.1 m. moles/kg.
0.5	1.55	1.21
1.0	0.9	0.56
2.0	0.75	0.49
3.5	0.75	0.38

EXAMPLE 15

7 - [D - (—) - 2,2 - dimethyl - 4 - (3,5 - dichloro - 4 - hydroxyphenyl) - 5 - oxo - 1-imidazolidinyl]cephalosporanic acid

A solution of (0.005 mole) of 7 - [D - (—)-α - amino - α - (3,5 - dichloro - 4 - hydroxyphenyl) - acetamido]cephalosporanic acid, 0.7 ml. (0.005 mole) of triethylamine in 50 ml. of methanol is obtained by stirring for 15 minutes at room temperature (22° C.). To this is added 50 ml. of acetone and stirring continued for 5 hours. The solution is then concentrated to an oil at 20° C. under reduced pressure. Twenty-five ml. of water and 50 ml. of ethyl acetate is added and the pH adjusted to 3 with 40% phosphoric acid. The aqueous layer is saturated with NaCl and the mixture shaken. The ethyl acetate layer is separated and dried briefly over sodium sulfate, filtered and concentrated to dryness at 20° C. under reduced pressure. The resulting solid precipitate is removed by ether trituration and filtration. After drying over phosphorus pentoxide under vacuum there is obtained the product 7 - [D - (—)-2,2 - dimethyl - 4 - (3,5 - dichloro - 4 - hydroxyphenyl) - 5 - oxo - 1 - imidazolidinyl]-cephalosporanic acid. Infrared and NMR spectra are consistent with the structure.

This product is found to inhibit *Staphylococcus aureus* Smith at a concentration of 2.5 mcg./ml., *Streptococcus pyogenes* at a concentration of 0.08 mcg./ml., *Staphylococcus aureus* BX—1633—2 (a strain resistant to benzylpenicillin at a concentration of 6.2 mcg./ml., *Escherichia coli* Juhl at a concentration of 12.5 mcg./ml., *Salmonella enteritidis* at a concentration of 1.6 mcg./ml., and *Diplococcus pneumoniae* at a concentration of 1.2 mcg./ml.

EXAMPLE 16

7 - [D - (—) - α - amino - α - (3,5 - dichloro - 4 - hydroxyphenyl) - acetamido]-decephalosporanic acid

When in Example 13, 7 - aminocephalosporanic acid is replaced by an equimolar amount of 7 - aminodecephalosporanic acid there is obtained 7 - [D - (—) - α - (t - butoxycarbonylamino) - α - (3,5 - dichloro - 4 - hydroxyphenyl) - acetamido] - decephalosporanic acid. Substitution in Example 14 of an equimolar amount of this compound for 7 - [D-(—) - α - (t - butoxycarbonylamino) - α - (3,5-dichloro - 4 - hydroxyphenyl) - acetamido]-cephalosporanic acid produces the product 7-[D - (—) - α - amino - α - (3,5 - dichloro-4 - hydroxyphenyl) - acetamido] - decephalosporanic acid.

This product is found to inhibit *Staphylococcus aureus* Smith at a concentration of 0.001 per cent by weight.

EXAMPLE 17

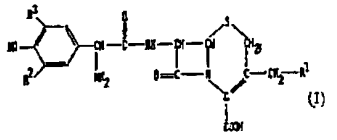
7 - [D - (—) - 2,2 - dimethyl - 4 - (3,5-dichloro - 1 - hydroxyphenyl) - 5 - oxo - 1-imidazolidinyl] - decephalosporanic acid

When in Example 15, 7 - [D - (—) - α-amino - α - (3,5 - dichloro - 4 - hydroxyphenyl) - acetamido] - cephalosporanic acid is replaced by an equimolar amount of 7 - [D-(—) - α - amino - α - (3,5 - dichloro - 4 - hydroxyphenyl) - acetamido] - decephalosporanic acid there is obtained the product 7 - [D-(—) - 2,2 - dimethyl - 4 - (3,5 - dichloro-4 - hydroxyphenyl) - 5 - oxo - 1 - imidazolidinyl] - decephalosporanic acid.

This product is found to inhibit *Staphylococcus aureus* Smith at a concentration of 0.001 per cent by weight.

WHAT WE CLAIM IS:—

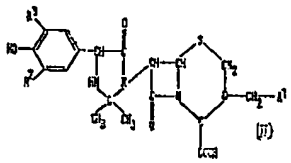
1. A compound of the formula



(I)

wherein R^1 is hydrogen or acetoxy and R^2 and R^3 are hydrogen or chloro.

2. A compound of the formula



wherein R^1 is hydrogen or acetoxy and R^2 and R^3 are hydrogen or chloro.

3. 7 - [D - (—) - α - Amino - α - (3,5-dichloro - 4 - hydroxyphenyl) - acetamido] cephalosporanic acid.

4. 7 - [D - (—) - α - Amino - α - (3,5-dichloro - 4 - hydroxyphenyl) - acetamido] decephalosporanic acid.

5. 7 - [D - (—) - 2,2 - Dimethyl - 4-(3,5 - dichloro - 4 - hydroxyphenyl) - 5 - oxo-1 - imidazolidinyl] cephalosporanic acid.

6. 7 - [D - (—) - 2,2 - Dimethyl - 4-(3,5 - dichloro - 4 - hydroxyphenyl) - 5 - oxo-1 - imidazolidinyl] decephalosporanic acid.

7. 7 - [D - (—) - α - Amino - α - (3-chloro - 4 - hydroxyphenyl)acetamido] - cephalosporanic acid.

8. 7 - [D - (—) - α - Amino - α - (3-chloro - 4 - hydroxyphenyl)acetamido] - decephalosporanic acid.

9. 7 - [D - (—) - 2,2 - Dimethyl - 4 - (3-chloro - 4 - hydroxyphenyl) - 5 - oxo - 1-imidazolidinyl] cephalosporanic acid.

10. 7 - [D - (—) - 2,2 - Dimethyl - 4 - (3-chloro - 4 - hydroxyphenyl) - 5 - oxo - 1-imidazolidinyl] decephalosporanic acid.

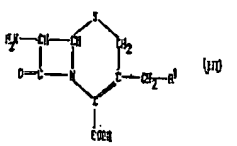
11. 7 - [D - (—) - α - Amino - α - (4-hydroxyphenyl) - acetamido] cephalosporanic acid.

12. 7 - [D - (—) - α - Amino - α - (4-hydroxyphenyl) - acetamido] decephalosporanic acid.

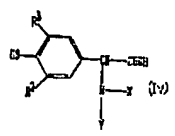
13. 7 - [D - (—) - 2,2 - Dimethyl - 4 - (4-hydroxyphenyl) - 5 - oxo - 1 - imidazolidinyl] cephalosporanic acid.

14. 7 - [D - (—) - 2,2 - Dimethyl - 4 -(4-hydroxyphenyl) - 5 - oxo - 1 - imidazolidinyl] decephalosporanic acid.

15. A process for the preparation of a compound of the formula I defined in claim 1, or a nontoxic, pharmaceutically acceptable salt thereof, which process comprises (a) acrylating a compound of the formula



wherein R^1 is as defined above, or a carboxylic acid salt thereof, with an acid of the formula



or an acylating derivative thereof, wherein —NXY is a protected amino group in which X is hydrogen and Y is t - butoxycarbonyl, benzyloxycarbonyl or 2,2,2 - trichloroethoxycarbonyl, or a like protecting group, or X and Y when taken together represent the 2 - hydroxy - 1 - naphthylmethylene group, or a like protecting group, in an inert solvent, at a temperature below 0° C., and (b) subsequently removing the protecting group to produce the compound of formula I, or a nontoxic, pharmaceutically acceptable salt thereof.

16. A process as claimed in claim 15, wherein the acylating agent is a mixed acid anhydride of the acid of formula IV.

17. A process as claimed in claim 15 or claim 16, wherein the protecting group is t-butoxycarbonyl which is subsequently removed by treatment with formic acid.

18. A process as claimed in claim 15 or claim 16, wherein the protecting group is benzyloxycarbonyl which is subsequently removed by catalytic hydrogenation.

19. A process as claimed in claim 15 or claim 16, wherein the protecting group is 2,2,2-trichloroethoxycarbonyl which is subsequently removed by treatment with zinc dust in glacial acetic acid.

20. A process as claimed in claim 15 or claim 16, wherein the protecting group is 2-hydroxy - 1 - naphthylmethylene which is subsequently removed by acid hydrolysis.

21. A process as claimed in any one of claims 15 to 20, wherein the inert solvent is tetrahydrofuran, dimethylformamide, methylene chloride, diethyl ether, acetone, chloroform, methyl isobutyl ketone, ethyl acetate, or a dimethyl ether of ethylene glycol and diethylene glycol.

22. A process as claimed in any one of claims 15 to 21, wherein the acylating step is carried out at a temperature of —25° C. or below.

23. A process as claimed in claim 15 and substantially as hereinbefore described with reference to any one of Examples 2, 5, 6, 9, 11, 14 and 16.

24. A compound as claimed in claim 1 whenever produced by a process as claimed in any one of claims 15 to 23.

25. A process for the preparation of a compound of the formula II as defined in claim 2, which process comprises reacting a compound of the formula I as claimed in any one of claims 1, 3, 4, 7, 8, 11, 12 or 24 with acetone,

at a pH of from 5 to 9, and at a temperature of from −20° C. to 50° C.

26. A process as claimed in claim 25, wherein the acetone is present in a molar excess.

27. A process as claimed in claim 25 or claim 26, wherein the reaction is carried out at a pH of from 7 to 9.

28. A process as claimed in claim 25 and substantially as hereinbefore described with reference to any one of Examples 3, 7, 10, 12, 15 and 17.

29. A compound as claimed in claim 2 whenever produced by a process as claimed in any one of claims 25 to 28.

30. A pharmaceutically acceptable non-toxic salt of a compound as claimed in any one of claims 1 to 14, 24 or 29.

31. A pharmaceutical composition comprising a compound as claimed in any one of claims 1 to 14, 24 or 29, or a salt as claimed in claim 30, and a carrier or diluent.

32. A pharmaceutical composition in unit dosage form containing 125 or 250 or 500 mgm. of a compound as claimed in any one of claims 1 to 14, 24 or 29, or a salt as claimed in claim 30, and a carrier or diluent.

33. A method of treating animals, including poultry but excluding man, for diseases caused by Gram-positive or Gram-negative bacteria, which method comprises administering to the animal an effective dose of a compound of formula I or formula II, or a salt thereof, or a pharmaceutical composition as claimed in claim 31 or claim 32.

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